Chapter 7
Seed Priming and Nano Priming Techniques as Tools to Alleviate Osmotic Stress in Legumes

Mohammed Mouradi, Mohamed Farissi, Ahmed Khadraji, Abdelaziz Bouizgaren, Ahmed Qaddoury, and Cherki Ghoulam

Abstract Salinity and drought are among the most influencing factors facing agricultural production. In many regions they cause up to 50 yield loss due to the secondary oxidative stress they create. The physiological reactions caused by oxidative stress adversely affect germination rate, plant growth, and development. Legumes, with their N2 fixing symbioses, developed various tolerance strategies to cope with these constrains, but the complexity of oxidative stress and climate change make it more difficult to maintain crop productivity. Seed priming may constitute an alternative as an easy, inexpensive, safe, and reliable technique for ameliorating germination under stress. It consists of inducing a particular physiological state in the plant via the treatment of the seeds with natural or synthetic agents before germination. Under unfavorable environmental conditions, seed priming allowed to restart the germination metabolism, thus improving the germination percentage and germination rate and reducing the germination time. Seed priming with nanoparticles (NPs) is a promising field of plant nanotechnology that can enhance osmotic stress tolerance by alleviating oxidative stress injuries in plants and install stress resistance in treated seedlings. Thus, this review will highlight the various potential benefits of NPs application as priming agents in the seeds of legumes and non-legumes, in some cases, through the comparison to the standard priming agents like polyethylene glycol (PEG), NaCl, and bioactive agents. Primed seeds 0showed low

M. Mouradi (✉) · M. Farissi
Unit of Biotechnology and Sustainable Development of Natural Resources (B2DRN), Sultan Moulay Slimane University (USMS), Beni Mellal, Morocco
e-mail: mohammed.mouradi@usms.ac.ma; mmouradi@outlook.com

A. Khadraji · A. Qaddoury · C. Ghoulam
Center of Agrobiotechnology and Bioengineering, Research Unit Labeled CNRST, Cadi Ayyad University (UCAM), Marrakesh, Morocco

A. Bouizgaren
Unit of Plant Breeding and Quality, National Institute for Agronomic Research (INRA), Marrakesh, Morocco

C. Ghoulam
AgroBioSciences (AgBS) Program, Mohammed VI Polytechnic University (UM6P), Benguerir, Morocco

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oxidative injuries due to the accumulation of osmoprotectants and osmotic adjustment stimulated by the variant priming agents including PEG, NaCl, etc. Bioactive priming agents like plant growth-promoting rhizobacteria (PGPRs), *Pseudomonas,* and *Trichoderma* are among many beneficial microorganisms used against biotic and abiotic stressors. Active NPs act in priming like biostimulants of salinity and drought resistance and enhanced water uptake. Seed germination and vigor, stimulate aquaporin (AQP) synthesis, photosynthesis, RuBisCo activity, antioxidant defense, nodulation in legumes, and nutrient uptake.

Keywords Salinity · Drought · Seeds · Priming · Nanoparticles · PGPRs

1 Introduction

Agriculture is facing many challenges due to climate change. Salinity and drought are the main abiotic factors to which plants are exposed during their life cycle. These two environmental constraints cause severe production loss by affecting growth, development and productivity of crops, especially in arid and semi-arid regions (Singh et al. 2015). Almost 20% of the world's arable land is affected by salinity, with an annual increase of 1 to 2% of land becoming affected. The presence of more salt in the soil than the plant needs disrupts its physiological, biochemical and metabolic processes (Xiong and Zhu 2002). Due to osmotic stress and ionic toxicity, salinity and water deficit induce the generation of reactive oxygen species (ROS), inhibit seed germination, reduce photosynthetic activity, disrupt membrane stability, and ionic balance and lipid metabolism (Farissi et al. 2018; Muchate et al. 2016; Aqtbouz et al. 2016; Zargar et al. 2017; Mouradi et al. 2018).

Various methods are used to improve plants' tolerance to abiotic stress. Selection of resistant varieties, natural crossings and genetic engineering are the main techniques to improve plants' tolerance to salt and water stresses (Jisha et al. 2013). Recently, 'seed priming' a pre-sowing treatment has been developed as a simple, effective, ecological, and natural method for improving plants' resistance to abiotic and biotic stresses (Bhanuprakash and Yogeesha 2016; Conrath et al. 2015). It consists of partially hydrating the seeds with natural or synthetic agents to proceed the pre-germinative events and prevent the radicle emergence in the imbibition phase. This creates a particular physiological state (plant tolerance memory) in the seed that can impact the plant tolerance in later growth stages (Abid et al. 2018; Chen and Arora 2013). When primed with the suitable agent, dry seeds accumulate more osmoprotectants and compatible solutes like proteins, glycine betaine, and sucrose, responsible of osmotic adjustment under salinity and drought constraints. It has been reported that this technique could improve seed germination, growth, photosynthesis, mineral and water nutrition, and the antioxidant system of plants (Lahrizi et al. 2021; Tounekti et al. 2020; Sen and Puthur 2020; Llorens et al. 2020; Parveen et al. 2019; Yusefi-Tanha et al. 2019).

Many nanomaterials, especially nanoparticles (NPs), belong to nanotechnology and have the potential to contribute as a new technological solution for agriculture problems. Many of them have proven their effectiveness when applied to plants for the protection from phytopathogens, plant nutrition amelioration and inducing resistance to abiotic stressors (Nayana et al. 2020; Maswada et al. 2020; Kumar et al. 2020). It has been reported that the binding proportion between seeds and NPs agents in nanopriming was found to be high compared to other priming agents like PEG, water and vitamins (Mahakham et al. 2017; Anand et al. 2019). The use of NPs and nanofetilizers as priming agents can strongly contribute to pest control, plant nutrition amelioration, and ecofriendly production methods. The NPs in seed priming can also act as biostimulants, improving seed germinative metabolism, plant growth, and activators of many signaling pathways. These effects depend on the size and the properties of the NPs applied to the seed. Researchers are exploring avenues for reducing fertilizer requirements by tweaking the seed metabolism through growth booster molecules or seed priming agents and using different nanoparticles as fertilizers.

The aim of this review is to provide an update concerning the potential applications of seed priming and nanopriming techniques with NPs and plant growth promoting rhizobacteria (PGPR) in legumes with their N_2 fixing symbioses and other plant species for mitigating the climate change effects and particularly salinity and drought constraints.

2 Seed Priming Techniques and Utilization in Legumes

One of the important challenges seed physiologists face is the selection of the priming medium. Seed priming has been reported to be one of the most widely used techniques to improve the tolerance of plants to abiotic stresses. This technique consists of inducing a particular physiological state in the plant by treating the seeds with natural or synthetic agents before germination (Lutts et al. 2016). Under unfavorable environmental conditions, seed priming helped restart germinal metabolism, thereby improving germination parameters as germination percentage, its rate and germination time (Pradhan et al. 2017; Lemmens et al. 2019).

Various seed priming techniques, including hydropriming, halopriming, osmopriming, chemopriming, biopriming, priming with growth hormones, etc., have been reported for their positive effects in improving plants' tolerance to some abiotic stresses like salinity and drought (Mouradi et al. 2018; Khadraji et al. 2020; Lahrizi et al. 2021) (Table 1). Although the role of seed pretreatment in improving seed and plant emergence has been reported by several authors (Khadraji et al. 2017, Mouradi et al. 2016b), the most suitable type of pretreatment mainly depends on the plant species and the type of stress to which the plants are exposed (Paparella et al. 2015).

Table 1 Techniques of seed priming, priming agents, and main effects

Priming technique	Plant species	Priming agent and concentrations	Abiotic stress	Seed germination and Agro-physiological effects	Citation
Halopriming (NaCl)	*Cicer arietinum* L	1 and 1.8 $g.L^{-1}$ NaCl	Drought	• Increases germination rate • Highest mean germination time and time to 50% germination (T_{50})	Khadraji et al. (2020)
Halopriming (NaCl)	*Medicago sativa* L	200 mM NaCl for 24 hours	Salinity	• Germination success (89.17%), • Germination velocity (index value of 14.9)	Farissi et al. (2016)
Osmopriming (PEG_{6000})	*Cicer arietinum* L	−0.5 and −0.7 MPa	Drought	• Improves germination performances • Increases seedling vigor index, growth and plant height • Enhanced the activity of peroxidase, catalase and reduced the malonyldialdehyde content	Khadraji et al. (2017)
	Medicago sativa L	−0.6 MPa	Drought	• Increases growth, leaf area, and nodulation • Enhance the photosystem II (PSII) efficiency, and relative water content • Improves Nitrogen fixation, P and K^{+} uptake	Mouradi et al. (2016a)

(continued)

Table 1 (continued)

Priming technique	Plant species	Priming agent and concentrations	Abiotic stress	Seed germination and Agro-physiological effects	Citation
	Medicago sativa L	−0.6 MPa	Drought	• Higher germination rate and growth • Enhance the activity of peroxidase (PO) and catalase (CAT) • Reduce the malonyldialdehyde (MDA) content and the electrolyte leakage	Mouradi et al. (2016b)
Biopriming	*Medicago sativa* L	*Ensifer meliloti* (Rm1021) strains	Drought	• Significantly improved chlorophyll, and photosynthesis performance and stomatal conductance • Enhance cell membrane stability	Lahrizi et al. (2021)
	Vigna radiata L and *Cicer arietinum* L	*Bacillus cereus*	Salinity	Increasing seedling height, number and length of leaves, root, and shoot biomass	Chakraborty et al. (2011)
	Pisum sativum L	*Typha angustifolia*	Salinity	Adequate levels of osmotica (Proline, total soluble sugars, Potassium and Phosphorus) Increases Chlorophyll and carotenoid contents	Ghezal et al. (2016)

2.1 Osmopriming

Osmopriming consists of immersing the seeds in an osmoticum such as mannitol, sodium chloride, or polyethylene glycol (PEG) and has a positive effect on the enhancement of seeds germination and seedlings growth, especially under stress conditions (Farooq and Basra 2006; Farooq et al. 2009; Chen and Arora 2011). This pretreatment makes it possible to influence the development of the seedlings, by modulating the metabolic and biochemical activities during the reversible phase of germination, which gives the seed a significant germination potential and subsequently allows a certain tolerance level to various abiotic stresses (Khadraji et al. 2017; Mouradi et al. 2016a) (Fig. 1). Osmopriming is a simple technique for the successful germination of many species, nodulation, and N2 fixation capacity for legumes and their production under environmental stress through the acquisition of nutrients from poor soils (Mouradi et al. 2016b; Amooaghaie and Nikzad 2013). Studies on the germination of alfalfa and chickpea seeds primed with polyethylene glycol 6000 (PEG 6000) showed a higher germination rate and growth compared to untreated plants. The highest germination percentages reached 90.8% under severe stress (Mouradi et al. 2016b). Osmopriming treatment by PEG 6000 improved the activity of antioxidant enzymes (peroxidase and catalase), maintained membrane stability through limiting phospholipids peroxidation (reduced malonyldialdehyde content) and reducing electrolyte leakage under this stress. In general, germination success was positively correlated with peroxidase (PO) and catalase (CAT) activities and the degree of membrane stability in drought-tolerant populations (Khadraji et al. 2017; Mouradi et al. 2016b) (Table 1).

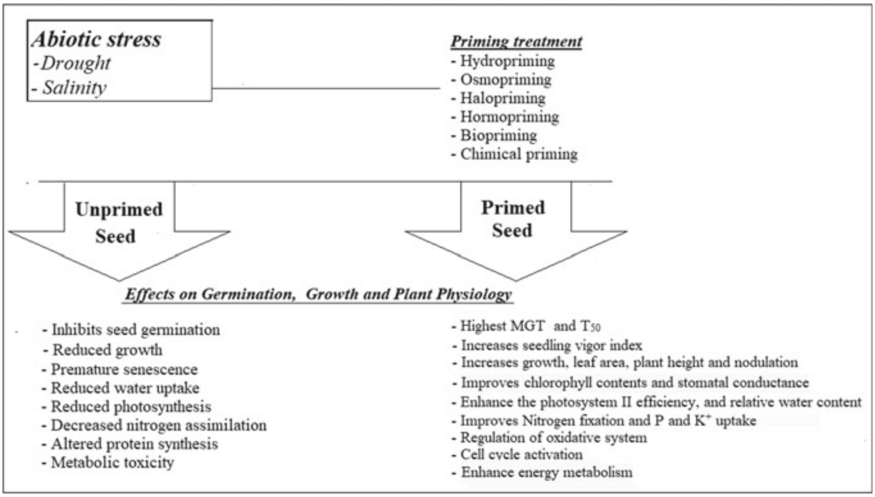

Fig. 1 Effects on germination parameters, growth, and plant physiology under different abiotic stresses

2.2 Halopriming

Halopriming is a technique of priming based on immersing seeds in different saline solutions ($CaCl_2$, $CaSO_4$, NaCl, etc.) that allows uniform germination of seeds, early emergence of seedlings, and an increase in biomass production, even under adverse environmental conditions (Khadraji et al. 2020; Jisha and Puthur 2014). This technique also induces the activation of enzymes involved in the breakdown and mobilization of reserves (Varier et al. 2010). The positive effects can continue even at the time of flowering and pod formation (Zare et al. 2011; Giri and Schillinger 2003). Several authors have explained the rapid and synchronized germination in the case of halopriming by activation of pre-germination processes, which promote quantitative and qualitative biochemical modifications at the level of the seed (Maroufi et al. 2011; Varier et al. 2010). Halopriming induces membrane repair (Jowkar et al. 2012) as well as the activation of endo-β-mannase (Varier et al. 2010) and more generally the increased activity of antioxidant enzymes (Ahmad et al. 2012). On the other hand, Varier et al. (2010) have explained the beneficial effects of halopriming on growth by an acceleration of nuclear replication in the roots and leaves.

2.3 Biopriming

Biopriming is an advanced treatment of seeds with biological means which allows both hydration and inoculation of the seeds by beneficial microorganisms before sowing, for improving the viability and vigor of the seeds as well as their germination, in particular under unfavorable conditions (Lahrizi et al. 2021; Mouradi et al. 2018). This technique also represents a kind of biocontrol through the use of microorganisms antagonistic to bacteria and phytopathogenic fungi in the soil by coating the seeds (Mahmood et al. 2016). The term biopriming was first introduced by Callan et al. (1990), where they applied a layer of biological primer on sweetcorn seeds with the fungi *Trichoderma asperellum* and *Trichoderma harzianum* and immersed them in lukewarm water (35–40% moisture content) for imbibition. In general, the goal of this treatment is to introduce beneficial microbes into the soil environment, followed by conventional inoculation (Reddy 2012). It allows uniform seed germination, viability, plant growth and finally improves crop yield. Most importantly, this ecofriendly approach protects seeds and plants from soil-borne pathogens mainly at the early stage of plant development (Lahrizi et al. 2021; Singh et al. 2016) (Fig. 1). Some biocontrol agents used with the seeds, like *Pseudomonas fluorescens* and *Clonostachys rosea,* have been shown to be able to colonize the rhizosphere, providing solubilized minerals to plants beyond the germination and seedling stages (Bennett and Whipps 2008). This depends mainly on the photosynthates exudation along with the root mucilage. This includes organic acids, amino acids, and carbohydrates, mandatory for microbial rhizosphere and root colonization (Reddy 2012). The main concern of this technique is related to the viability of the microbial agent on the surface of the seed. It has been demonstrated that PGPR strains keep on multiplying on the surface and in the spermosphere of seeds even before sowing (Mirshekari 2012; Reddy 2012). The bacterial survival depends on the species, soil proprieties, nutrients, competition with pathogens and other microbes, and water availability. Lahrizi et al. (2021) have demonstrated that plants raised from bioprimed seeds with rhizobia showed significant improvement of photosystem II performance, leaf relative water content, nodulation, and membrane integrity under water deficit (Table 1). Reddy (2012) reported that several microorganisms like *Trichoderma*, *Pseudomonas*, *Azotobactor*, *Azospirillum*, and *Agrobacterium,* when used as priming agents, can improve drought tolerance. Mirshekari (2012) demonstrated that seed biopriming with *Typha angustifolia* improved the salinity resistance in *Pissium sativum* L. Primed seeds showed better germination into seedlings under salinity by modulating membrane integrity. Photosynthesis, sugar metabolism and ionic hemostasis were also ameliorated in bioprimed *P. sativum* seedlings (Ghezal et al. 2016).

3 Nanoparticles (NPs) for Seed Priming for Legumes

Seed nano priming is a technology of seed treatment that uses nanomaterials for seed priming (Griffin et al. 2017). The particularity of this technique is that the priming solution is a suspension of nano formulations, mainly nanoparticles (NPs) of 1–100 nm. The nanoparticles are the building blocks of the nanotechnology. NPs are abundant in nature from inorganic ash, soot, sulfur, and mineral particles found in the air or in wells, to sulfur and selenium nanoparticles produced by many bacteria and yeasts (Buzea et al. 2007; Griffin et al. 2017). These are formed by many natural processes such as volcanic eruptions (silicate and iron compounds), forest fires (carbon nanotubes), erosion plants and animals shedding (selenium and tellurite) and photochemical reactions (silver NPs) (Griffin et al. 2017; Buzea et al. 2007; Bartlett et al. 2016). The NPs are recognizably different to the bulk materials for their small size and large surface area (Hong et al. 2013). Other differences are related to the physical strength, chemical reactivity, electrical conductivity, magnetism, and also optical effects (Hong et al. 2013). These proprieties allow them to be used in several industrial domains including food production and agriculture to reduce production cost. Several studies showed that nanoparticles have a great potential to be used in agriculture like nanosensors, with phytohormones, food additives, genetic improvement, for drugs, nano pesticides and nanofertilizers (Hong et al. 2013). The main factors adjusting the effect on plants depend on the plant species, the NPs intrinsic proprieties and concentration, the interaction time, and the interaction between living environment and plant (Miralles et al. 2012).

In seed nano priming, the NPs may or may not be taken up by the seed. Most of the nano priming techniques employ nano suspensions where the majority of NPs is retained in the seed surface or coat (do Espirito Santo Pereira et al. 2021). The seed nano priming can be used with seed coating with fungicides and insecticides in order to protect the crop from biotic ravagers or with biostimulants to improve tolerance

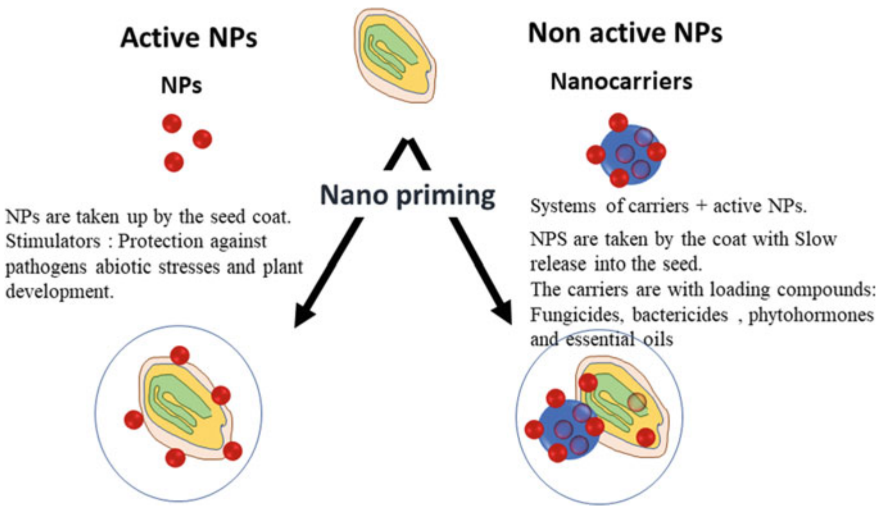

Fig. 2 Seed nanopriming. Active nanoparticles (NPs), systems that can be taken directly by the seed coat. They are generally plant growth and development stimulators and Nonactive NPs are systems of carriers providing a slow release of NPs during germination and seedlings growth and can be loaded by active compounds like fungicides, bactericides etc. (do Espirito Santo Pereira et al. 2021)

to abiotic stressors (Nayana et al. 2020; Bayat et al. 2020). In nano priming, we can distinguish two types of NPs, active or nonactive NPs (Fig. 2). The active NPs are particles that will be taken by the seed and retained in the seed coat after application. The nonactive particles will be used as nanocarriers for the active NPs when applied to the seed. The active NPs have been shown to ameliorate seed germination, and act as defense mechanisms against pathogens and environmental stresses (Chandrasekaran et al. 2020; Agathokleous et al. 2019). The system of carriers and active NPs is characterized by the slow release of the active NPs to the seed during germination. Both nano priming systems can be applied to seeds in order to provide protection during storage, improve germination, germination synchronization, and plant growth, as well as to increase the resistance to abiotic or biotic stress conditions (Chandrasekaran et al. 2020; Agathokleous et al. 2019). Active NPs can be characterized as direct stimulators of plant growth and development through activating biological effects and responses against stressors (Rizwan et al. 2019; Mishra and Singh 2015). Nanocarriers are systems of NPs that can be active itself or when loaded by other bioactive or synthetic compounds providing a slow release over time in the seed coat (do Espirito Santo Pereira et al. 2021; Kumar et al. 2020). Metallic and nonmetallic NPs can be used as active NPs priming suspension (Table 1). They have a direct effect on the seed germination and seedling growth. Many biopolymeric NPs can be used for the slow-release system. The biopolymers (more than 100 nm) are made from polysaccharides, lipids, and proteins and can be loaded by many substances of essential oils, pesticides, phytohormones, and fertilizers. For nano priming, alginate, cellulose, chitosan, and lipid NPs can be used to modify plant metabolism or against pathogens (do Espirito Santo Pereira et al.2021; Nayana et al. 2020; Kumar et al. 2020; Bayat et al. 2020).

3.1 Important Effects on Seed Germination

Nanomaterials can be applied to seeds (nanopriming) for the induction and improvement of seed germination, seed protection during storage, enhancement of plant growth, and the resistance to abiotic or biotic stressors (do Espirito Santo Pereira et al. 2021). Mahakham et al. (2017) investigated the effect of Ag NPs with *Citrus hystrix* leaf extracts to improve the germination of non-legume *Oryza sativa* seeds. The results demonstrated an enhancement of seed water uptake, α-amylase activity, and starch hydrolysis and produced more reactive oxygen species (ROS). Also, Maroufi et al. (2011) demonstrated that titanium (TiO_2, 0.02%) NPs improved germination and seedling growth of *Vigna radiata*. Carbon nanotubes also accelerated seed germination, growth rates as well as seedling vigor in tomato (Mondal et al. 2011). Bayat et al. (2020) demonstrated that the combination of iron and zinc NPs as seed priming accelerated the emergence of cotyledons in red beans and common beans cultivars. This may perhaps be due to the role of iron and zinc elements in the functional changes of different enzymes, which in turn causes positive synergistic effects on the bean plants. Furthermore, Hussain et al. (2019) reported that silicon (Si NPs) application as seed priming increased biomass and yield while reducing oxidative stress in wheat plants subjected to cadmium stress. Abdel-Aziz and Rizwan (2019) reported that silver (Ag NPs) increased growth of *Vicia faba* seedlings, photosynthesis, chlorophyll content, and starch accumulation. In the same sense, Zmeeva et al. (2017) showed that Si NPs improved plant height and tillers number, yield, fresh and dry root mass, plant transpiration, chlorophyll and carotenoids and photosynthetic pigments in *Medicago sativa*. It has been demonstrated also that silver NPs, used as priming suspension, may protect the seed from bacteria and fungi while silica NPs had the potential to improve the leaves mechanical strength, light absorption, enhance photosynthesis capacity, plant growth and endurance of plant organs and also reduce transpiration (Abbasi Khalaki et al. 2021). Iron NPs application control antioxidant activities and the functioning of phytohormones to enhance plant biomass while that of Titanium NPs helps to increase seed water absorption and boost vigor of old seeds (Abbasi Khalaki et al. 2021).

The majority of nanoparticles used with the seed nano priming for legumes are.

3.2 Seed Nanopriming and Oxidative Stress Tolerance

With their smaller size and higher effectivity, the NPs can help reduce the required quantities of chemical pesticides and fertilizers. Inside the plants and via the active transport system through xylem, NPs can change their structure and form ion complexes with other molecules or nutrients. The NPs can modulate the enzymatic activities related to the secondary oxidative stress, induced under osmotic stress, and activate the stress defense mechanisms. NPs can, at moderate levels, induce the generation of ROS, which constitute signaling pathways for transcription of different genes during the germination, and regulate secondary metabolites in germinated seedlings related to stress tolerance. These beneficial effects depend on the size and concentrations of the NPs and their physicochemical proprieties, mode of application, and the plant species (Fig. 3).

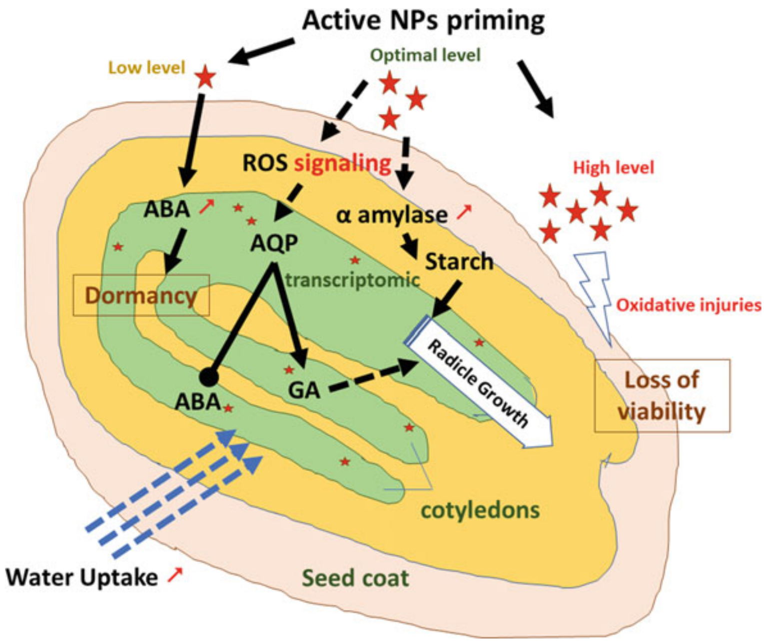

Fig. 3 Supposed model by Chandrasekaran et al. (2020) and do Espirito Santo Pereira et al. (2021) for the events occurring in nanoprimed seeds compartments by the action of active NPs. At low levels active NPs taken by the seed coat increased abscisic acid (ABA) synthesis and seed dormancy installation. At optimal level NPs induced ROS activation and signaling regulation of the gibberellic acid (GA) and ROS scavenging enzymes. Undetermined involvement of GA in internalization as well as NPs transport from seed coat to endosperm. Factors involved in sugar signaling responses, α amylase activity after NPs adhesion to radicle growth is indicated. ROS signaling of aquaporins (AQP) and increasing water uptake after NPs adhesion. At high level, NPs cause oxidative injuries through high levels of ROS and loss of the seed viability

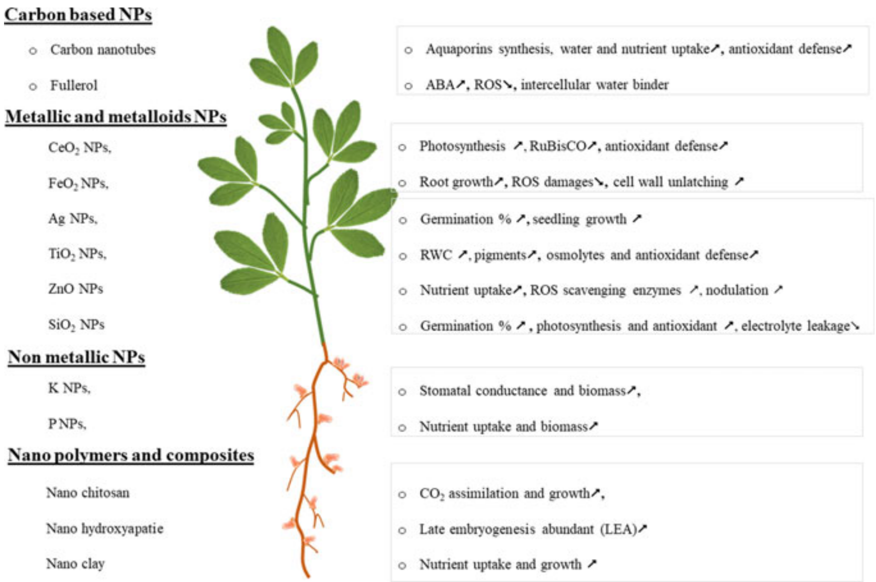

Fig. 4 NPs priming variant roles in overcoming drought stress in legumes and other plants (do Espirito Santo Pereira et al. 2021; Maswada et al. 2020; Agathokleous et al. 2019)

Generally, the beneficial effect of NPs under stress is clear at low doses and causes a low response stimulation "hormesis" under stress (Agathokleous et al. 2019). For example, in some non-legume species, carbon nanotubes application at 50 mg/l can stimulate drought tolerance by enhancing water uptake and the reduction of oxidative injury of *Hyoscyamus niger* L. while at high dose it causes cell injury (Hatami et al. 2017). Xiong et al. (2018) demonstrated that seed nanopriming with Fullerol represses ROS generation in *Brassica napus* L. by regulating the activation of non-enzymatic and enzymatic antioxidant compounds and also the ABA accumulation. The metallic NPs have also been demonstrated to be effective against drought in many species. Seed priming with iron NPs improved growth, photosynthesis, and photosystem II in sorghum plants (Maswada et al. 2018). Bayat et al. (2020) reported that soaking legumes seed with iron oxide NPs 4% for 3 hours enhanced growth and development of red beans (Table 2). Cao et al. (2018) demonstrated that Cerium oxide NPs improved biomass, water productivity, photosynthesis, and Rubisco activity in soybean plants subjected to different drought conditions (Fig. 4). Seyed Sharifi (2016) reported that ZnO NPs application next to biofertilizers improved nodulation in *Glycine max* L.. Mohaddam et al. (2017) reported that the combination of ZnO and Ag NPs enhanced nodulation in legume-rhizobium symbioses. This demonstrated that the beneficial effects of seed priming are conserved even at post germinative plant stages with markable changes in several physiological and biochemical responses in legumes and non-legumes (Mouradi et al. 2016a; Maswada et al. 2020). Seed priming with colloidal Molybdenum (Mo) NPs in the presence of microbial preparation stimulates root nodulation, symbiotic system, and antioxidant defense in chickpea under stress conditions (Taran et al. 2014). TiO_2 NPs can also stimulate plant growth in mung bean shoots and root length, nodulation, and promotes microbes in the rhizosphere (Raliya et al. 2015). These NPs have been reported enhancing the many crop performances and stress tolerance including soybean (*Glycine max* L.). this through enhancing chlorophyll content, photosynthesis and nutrient uptake (Andersen et al. 2016).

The effect of seed nanopriming on non-legumes like *Zea mays* L. and *Capsicum annum* L. under salinity stress has been investigated by Shah et al. (2021) and Ye et al. (2020). TiO_2 NPs (60 ppm) application mitigates salinity injuries in maize by maintaining leaf water content and inducing antioxidant enzymatic defense under salinity, 200 mM NaCl. Mn NPs at low concentrations (0.1, 0.5, 1 mg/L) improved the root growth (elongation) in salt-stressed seedlings of *C. annuum* L. (Ye et al. 2020). Mn NPs penetrates the seed coat, reduces the injuries of oxidative stress, and forms nanoparticle-corona complexes. This may play an important role in installing late salt tolerance in *C. annuum* L. (Fig. 5) (Ye et al. 2020). Shafiq et al. (2021) reported that Fullerenol at 80 nM improved ion uptake to reduce sodium toxicity and ameliorated biomass and grain yield in wheat plants under 150 mM NaCl. In legumes, nano silica (8 g/L) and gibberellic acid enhanced seed germination of pea, water uptake, ROS, and antioxidant in the seed under salinity (Chourasiya et al. 2021). Maroufi et al. (2011) reported that under salinity TiO_2 NPs (0.02%) significantly ameliorated germination percentage, seedling dry weight, and seedling vigor in *Vigna radiata* L. This nanopriming techniques can ameliorate seed germination performance and quality under stress in many ways including the activation of α-amylase activity, soluble sugars content, and stimulation of the activity of aquaporin channels increasing antioxidants to scavenge ROS, and the formation of nanopores to increase water uptake (do Espirito Santo Pereira et al. 2021).

3.3 Seed Nano Priming with PGPRs

PGPRs are root associated beneficial bacteria known for their ability to promote plant growth with direct or indirect mechanisms. Bacteria with direct mechanisms involve those related to nutrients mobilization like phosphate, zinc, iron, and sulfur, nitrogen-fixing symbioses and phytohormones production (Grobelak et al. 2015; Nayana et al. 2020). Indirect mechanisms involve protection against phytopathogens and enhancement of plant tolerance to abiotic stresses. The great variability of the PGPRs behavior is due to many factors like soil, plant species, and competitiveness with other microorganisms which is challenging their exploration as biofertilizers (Nayana et al. 2020).

In biopriming, PGPRs are applied as bacterial suspensions to the seeds, root surfaces, or directly in the rhizosphere. A consortium of bacteria has been proven to be more effective against indigenous microorganisms' competition in the soil then the

Table 2 Nanoparticles (NPs) utilized in seed nanopriming for legumes, their characteristics, the main effects on each studied species and stress conditions

Nano particle priming	Concentration and characteristics	Main effects	Legume species	Citation
Iron oxide	Soaked in 4% concentration and dried for 30 minutes in shade	Growth and development	Red beans	Bayat et al. (2020)
FeS_2 Nano-iron pyrite	100 µg/mL aqueous suspension of FeS_2 for 12 hours (overnight)	Biomass, number of leaves, root, and shoot length	*Medicago sativa* L	Das et al. (2016)
Zinc nanoparticles	–0.15%, size 20 nm spherical shape 40 and 60 nm elongated shape (3 hours) –0.006%, size 21.3 nm for 12 hours	Salinity resistance, increase of SOD, CAT, POD, and APX enzymes activities, photosynthetic pigments, organic solutes, as well as total phenols, ascorbic acid, and Zn over stressed plants alone	• *Phaseolus vulgaris* L • *Lupinus termis* L	Mahdieh et al. (2018), Latef et al. (2017)
Titanium NPs	2% for 24 hours	Increase in root and shoot length, lateral roots antioxidant enzymes	*Phaseolus vulgaris* L	Paul et al. (2020)
Silver nanoparticles (Ag NPs)	0.005% for 6 hours –0.000125% for 1.5 hours	Growth attributes and biomasses of bean seedlings chlorophyll contents, starch, and total carbohydrate contents • Biomass, plant height, number of nodules, Net photosynthesis intensity	• Broad bean • Green bean	Abdel-Aziz and Rizwan (2019), Prażak et al. (2020)
Platinum nanoparticles stabilized with poly (vinylpyrrolidone)	1 mM, size 3.2 nm, and spherical shape for 3 hours	Seed and seedling vigor, plant morphology, higher yield	*Pisum sativum* L	Rahman et al. (2020)

(continued)

Table 2 (continued)

Nano particle priming	Concentration and characteristics	Main effects	Legume species	Citation
Copper nanoparticle	0.1%, size 25 nm for 20 min	Seed and seedling vigor and biomass. High concentration inhibited seed germination	*Phaseolus vulgaris* L	Duran et al. (2017)
Silicon SiO_2 NPs	0.006% for 48 hours	Increased germination rate 20% of deteriorated seed, reduced the mean germination time (MGT)	*Glycine max* L	Mansouri Gandomani and Omidi (2017)
Chitosan and carbon nanotubes	• Chitosan 10% nanoparticles with size of 95 nm • Carbon nanotubes 10% with size of 40 nm for 3 hours • CsNPs 0.05%, size 20 nm for 3 hours	• Plant morphology ROS elevation • Seed germination, total polyphenols, antioxidant activities	*Phaseolus vulgaris* L Broad Beans	Zayed et al. (2017), Abdel-Aziz (2019)

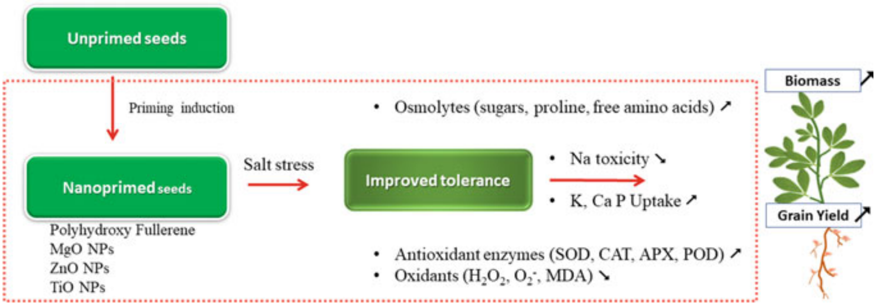

Fig. 5 Proposed Model of the effect of NPs priming on plants under salinity stress (Shafiq et al. 2021; Ye et al. 2020; Abou-Zeid et al. 2021)

single inoculation. The microbial consortium can be prepared with NPs for wider use due to their small amounts and great effects on plant growth and resistance (Fig. 6) (Nayana et al. 2020). In nature, root exudate can produce various nano size metallic formulation acting as plants bio stimulants in the rhizosphere. It is reported that Gold NPs with *Pseudomonas monteilii* enhanced indole acetic acid (IAA) production in the bacteria and improved probiotic effect in cowpea. ZnO NPs had been reported to also ameliorate nodulation, plant height, grain yield and weight in soybean (Seyed Sharifi

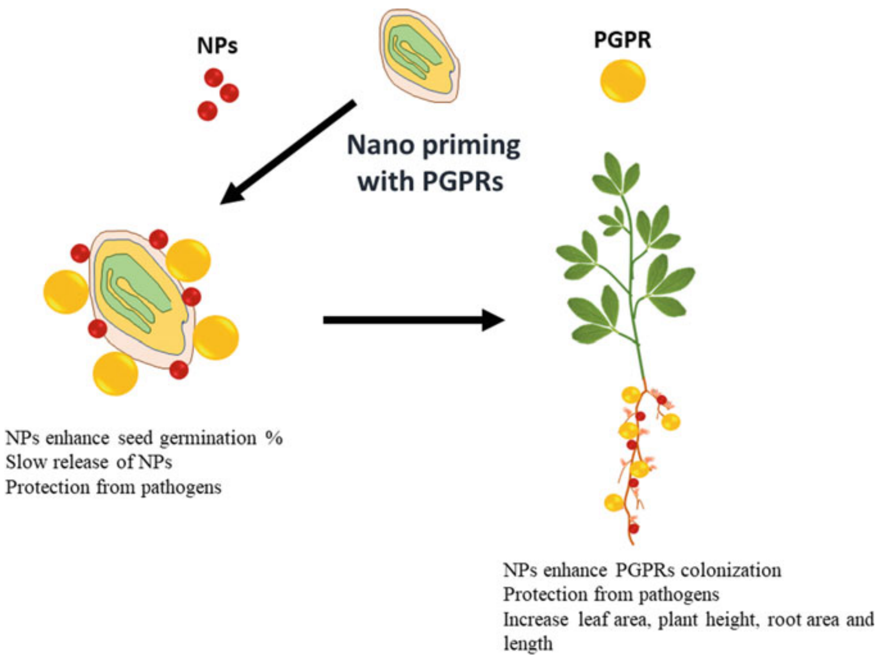

Fig. 6 Combined application effects of NPs and PGPRs (Nayana et al. 2020)

and Khoramdel 2015). In non-legumes, Karunakaran et al. (2013) reported that SiO NPs synthesized from rice ash with a consortium of *Bacillus*, *Pseudomonas,* and *Azotobacter* genera improved seed germination percentage of maize in comparison to conventional Si NPs. Hatami et al. (2021) reported that the application of SiO NPs (100 mg/L) as seed priming with *P. fluorescens* produced healthy seedlings of lemon balm with higher biomass, RWC, photosynthetic pigments and antioxidant activity, and lower membrane electrolyte leakage. The application of SiO NPs induced the appearance of micropores in the seed coat causing higher water uptake and healthier seedlings (Hatami et al. 2021).

4 Conclusion and Recommendations

Salinity and drought are the major factors facing agriculture production and food security in many regions around the world. The present chapter reveals that seed priming is a safe and easily applied technique for uniform and successful germination and establishments of legumes, especially under salinity and drought. Seed priming enhanced germination parameters under stress including germination percentage, mean germination time (MGT), germination rate, and seedlings growth by increasing antioxidants. Seed priming also ameliorated the N_2 fixing ability of legumes by enhancing nodulation, rhizobia root colonization, and nutrient uptake. According to the literature in this review, the application of NPs as nano priming agents is significantly more important in stimulating osmotic stress tolerance in legumes and other plant species than the standard priming agents like PEG, nutrients, and vitamins. NPs in seed priming can alleviate osmotic stress damages by inducing antioxidant defense, osmoprotectants, and ion balance in the seed and thus promote water uptake, seed germination, and seedling health. NPs can also stimulate aquaporins synthesis, photosynthesis, Rubisco activity, nodulation in legumes, and nutrient uptake. In addition, the application of NPs with PGPRs is a promising field of biofertilizers research. Further l studies will be needed to examine the interactions of treated plants with NPs and PGPRs consortia in order to increase osmotic stress tolerance in legumes.

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